STUDIES ON *GRACILARIA:* ECOLOGY OF AN ATTACHED POPULATION OF *GRACILARIA* SP. AT BARRACHOIS HARBOUR, COLCHESTER CO., N.S.*†

N. Bird
Atlantic Regional Laboratory,
National Research Council of Canada,
Halifax, N.S. B3H 3Z1

In situ observations were made on an attached population of *Gracilaria* sp. at Barrachois Harbour, Colchester Co., Nova Scotia. The growing season, characterized by water temperatures close to 20°, was less than 3 months. Sporelings transplanted to the field became reporductively mature within 6-10 weeks. Reproductive periodicity of the population was noted; release of tetraspores, first observed in late June, and release of carpospores, initiated at the end of July, continued until late September. Reproductive peak of the population occurred in early August. In contrast to free-floating populations, percentage of gametophytes and sporophytes was similar. Senecence was noted in late August. Plants overwintered as 'stump' plants that resumed growth upon increasing light and temperature in spring. Spore germination and survival of sporelings at low temperatures were demonstrated.

Introduction

Species of *Gracilaria* Greville are cosmopolitan in distribution, and a single species, *Gracilaria* sp (Chapman *et al* in press), is recorded from the Maritime provinces (C. Bird *et al* 1977a). *Gracilaria* sp is a common species of larger rhodophycean algae occurring in the lower Gulf of St. Lawrence with a biomass in excess of 4 $kg.m.^{-2}$ having been recorded (C. Bird *et al* 1977b). However, the distribution of this species is limited apparently to shallow, warm-water embayments (C. Bird *et al* 1977a; Edelstein *et al* 1967).

In the Maritime provinces, *Gracilaria* sp occurs largely as free-floating populations. The plants are large, often profusely branched and commonly 10-30 cm in length. These populations usually are stabilized either by entanglement amongst eel grass, *Zostera marina* L. var. *stenophylla* Aschers. & Graelsw., or entwined by byssal fibers of species of mussels (Goldstein 1974). However, in most populations a few attached specimens, with basal holdfasts, may be found on suitable substrata such as small rocks and shells.

Observations in the Maritime provinces and elsewhere in the world suggest that free-floating populations of species of *Gracilaria* are commonly infertile or asexual, and are maintained mainly through vegetative propagation. Contrariwise, attached populations are characterized by both asexual and sexual generations, and reproduction undoubtedly is mainly, if not exclusively, through spores (Causey *et al* 1946; Kim 1970; Simonetti *et al* 1970; Stokke 1957).

The occurrence of a wholly attached population of *Gracilaria* in Barrachois Harbour, Colchester Co. (C. Bird *et al* 1977a) provided an opportunity for detailed ecological observations in a habitat different from those in which this alga normally has been reported.

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Materials and Methods

An attached population of *Gracilaria* at the pond head of Barrachois Harbour, Colchester Co. was studied *in situ*, using SCUBA, at regular intervals over a two-year period, 1974 and 1975. For comparison, periodic examinations also were made on a predominantly free-floating population of this species at Mill River, Prince Co., Prince Edward Island.

Surface samples of water were obtained by filling plastic bottles held just beneath the surface of the water; subsurface samples by diving to the bottom before opening. Temperature of the water was recorded by inserting a thermometer into the bottle soon after collection; a hydrometer was used for salinity determinations.

The zone of *Gracilaria* was delimited by laying a horizontal transect across the channel at the mouth of the harbor. A study line of 90 m of polypropylene was laid parallel with the channel in the zone of *Gracilaria*, and anchored every 15 m with a concrete buliding block. Using randomly chosen positions on the line, length and density measurements of *Gracilaria* were taken by placing a 1-m^2 quadrat, constructed of 0.25-in, stainless-steel tubing, between these points. The quadrat was bisected with a rod of tubing allowing two divers to record simultaneously over an area of manageable size. Maximum length of plants was determined by holding a ruler against the base of the plant at substrate level. Total number of plants in the quadrat was regarded as density. Sanded, white Arborite and a leaded pencil were used to record observations.

Production of spores in *Gracilaria* during July, August, and September was determined by examining branches from plants in quadrats as described above. One branch was removed from each plant as close to the main axis as possible, and only plants above 3 cm tall and having more than one erect frond were sampled. Branches were collected in the upper half of a wide-mouth bottle, flamed at the cut surface to form a ridge over which a fine nylon mesh bag was held in place by a metal screw band.

Fertility of *Gracilaria* sp at Mill River was examined in early August, 1975. A 100 m marked transect line was laid perpendicular to the shore starting at the high water mark. At depths of approximately 1.0 and 2.0 m plants within a 0.5 m quadrat were collected for determination of fertility ratio. All plants with holdfasts within each quadrat were examined. In these samples a large number of detached plants was collected but only a handful were studied. In addition 6 quadrats were cleared parallel to the shore at a depth of 0.5 m.

Actual fertility ratios for Barrachois and Mill River populations of *Gracilaria* sp were determined in the laboratory by recording the number of plants that were male, cystocarpic, tetrasporic, or infertile. For the population at Barrachois, potential fertility ratios were obtained by incubating the remaining infertile branches in culture for periods up to 6 weeks. Conditions of incubation have been described previously (N. Bird *et al* 1977).

Transplantation experiments were carried out at Barrachois using sporelings of *Gracilaria* sp grown in laboratory culture (N. Bird *et al* 1977) on oyster or scallop shells having centrally drilled holes. These sporelings, with fronds about 10 mm long, were transplanted to the field. Shells were strung on cable (Northern Electric Station Z wire) spaced by knots in the wire or thick-walled rubber tubing. The cable was anchored to the study line, and suspended vertically by a small buoy so that sporelings were positioned approximately 0.5 m above the study line. The softer scallop shells proved unfavorable, since they wore easily where in contact with the cable. Shells with sporelings also were nailed to the substrate by 15-cm galvanized nails.

Results

Barrachois Harbour consists of a salt-marsh estuary with a soft, muddy bottom. A channel, approximately 3-5 m deep, depending on the state of the tide, runs through the middle of the estuary. It begins at the mouth of the harbour and extends for a considerable distance beyond the bridge (Fig. 1). The mean tidal amplitude at Barrachois is about 2 m, and the constriction in the harbor results in swift tidal currents. The harbor is covered with ice usually for about 4 months, late December to early April.

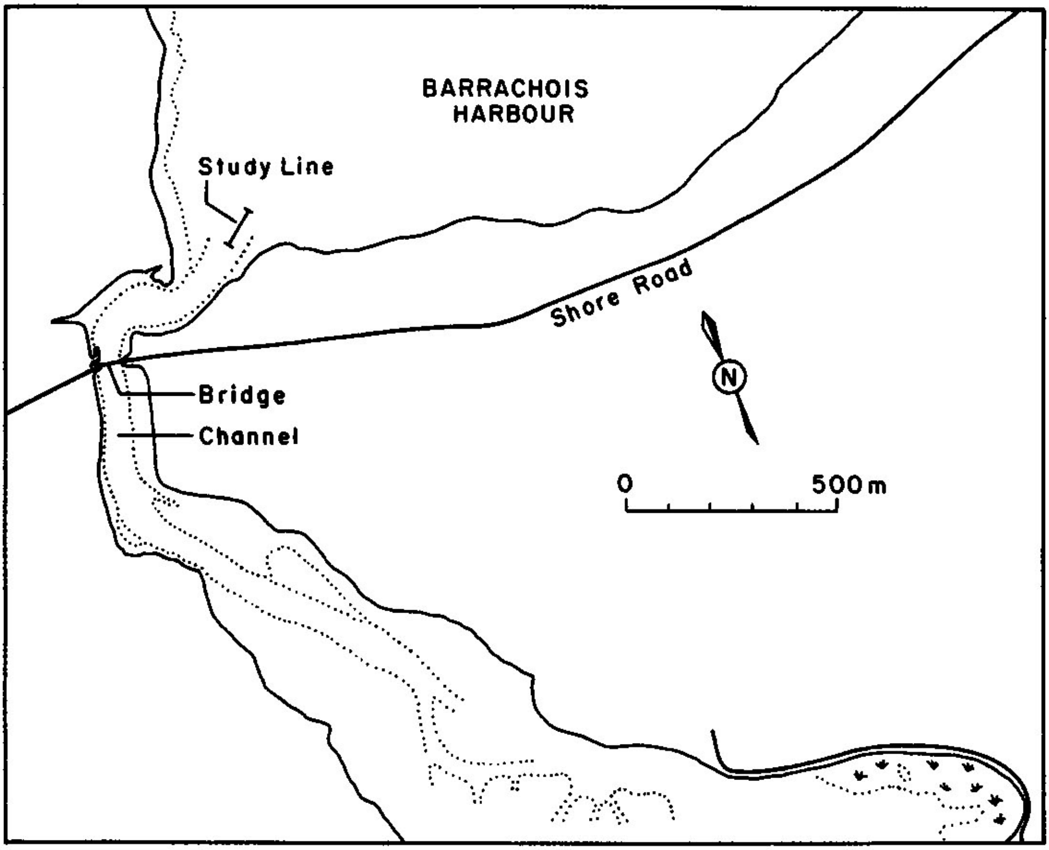

Fig. 1. Pond head of Barrachois Harbour, Colchester Co., N.S.

Vegetation was sparse except where gently sloping sides or shelves offered protection. On one side of the channel vegetation was divided into six distinct zones according to depth at 0.8 m below M.W.L. (Canadian Tide and Current Tables) (Fig 2). The "fine reds" - *Lomentaria baileyana* (Harv.) Farl., *Chondria tenuissima* (Good. et Wood.) C. Ag., *Griffithsia globulifera* Harv., and *Dasya baillouviana* (Gmel.) Mont. were present throughout the five lower zones, while remaining major plant species were restricted to a narrow range of depth. Upper boundary of the zone of *Gracilaria* merged with the lower boundary of the zone of *Z. marina* at a depth of 1.3 m. This 2- mwide zone of transition ended at a depth of 1.6 m, when *Z. marina* became scarce.

The bed of *Gracilaria*, consisting of a mud bottom scattered with rocks and shells suitable for attachment of plants, extended down the slope for 8 m and a depth of 1.8 m. Depths recorded in this zone throughout the study period varied as much as 2 m for tides ranging from 1.2 m below to 0.4 m above M.W.L. Depths recorded along the study line every 15 m ranged from 1.8 - 2.6 m for a tide of 0.5 m below M.W.L. Below this zone vegetation became sparse as the barren channel was neared. Except for the zone of *Z. marina* found at similar depths on both sides of the channel, the opposite side of the channel was barren (Fig 2).

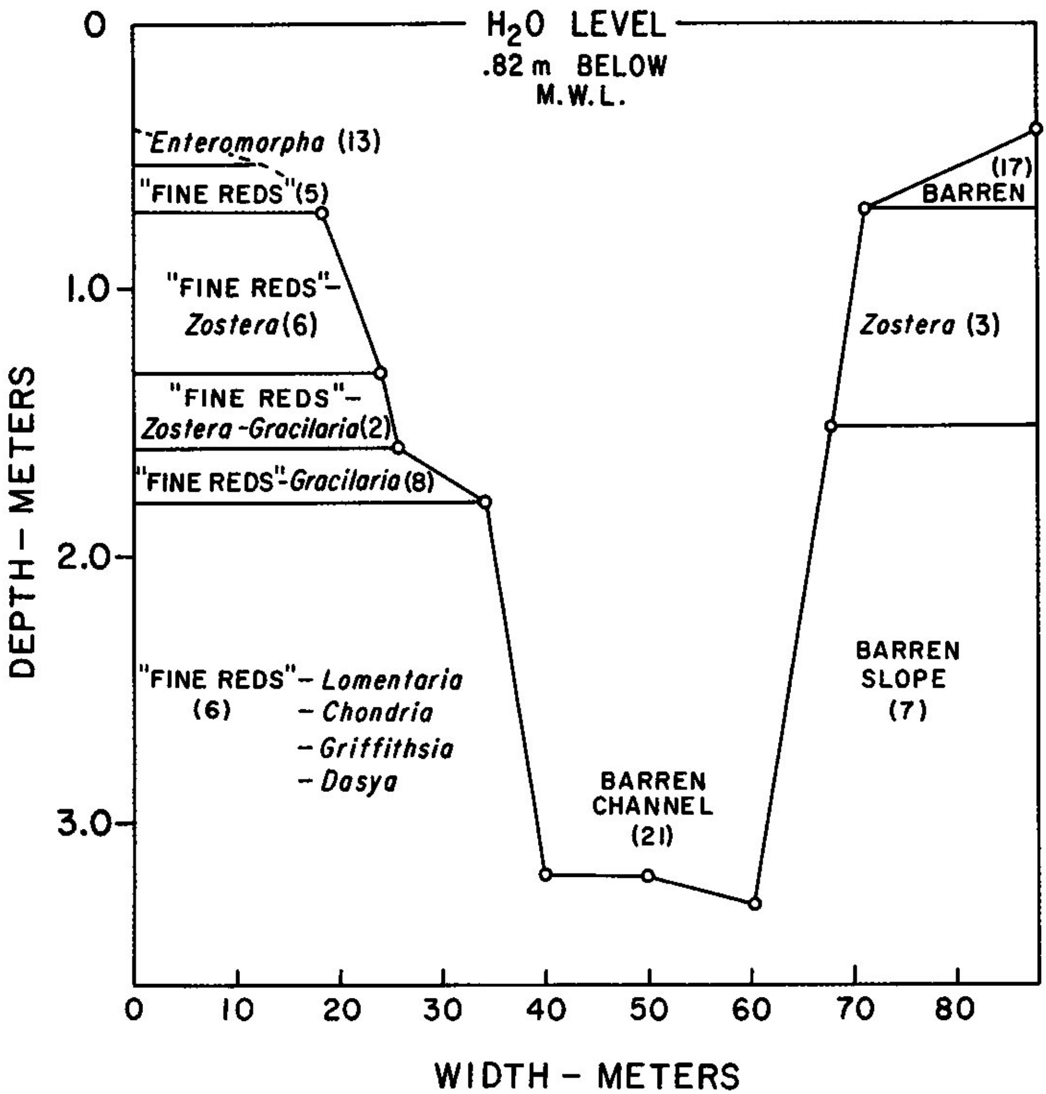

Fig. 2. Horizontal transect across channel at Barrachois showing vegetation zones in relation to depth. Widths (m) of zones are in parenthesis.

The zone of *Gracilaria* ran lengthwise, parallel to the channel (Fig 1). Progressing towards the bay, plants of *Gracilaria* sp became less frequent, being scattered amongst *Z. marina*; below the *Z. marina* zone, fine sand was prevalent.

Gracilaria sp was abundant about 70 m beyond the end of the study line, towards the bridge (Fig 1); in this area plants were found at depths of 0.5 m (1.2 m below M.W.L.). The zone ended as the side of the channel steepened. Estimated area of the zone of

Gracilaria sp was about 1000 m^2. The channel extended for a considerable distance beyond the bridge towards the salt marsh, but *Gracilaria* sp occurred only amongst large rocks near the concrete bridge supports.

Warmest water temperatures, commonly above 20° (Fig 3), occurred from July til

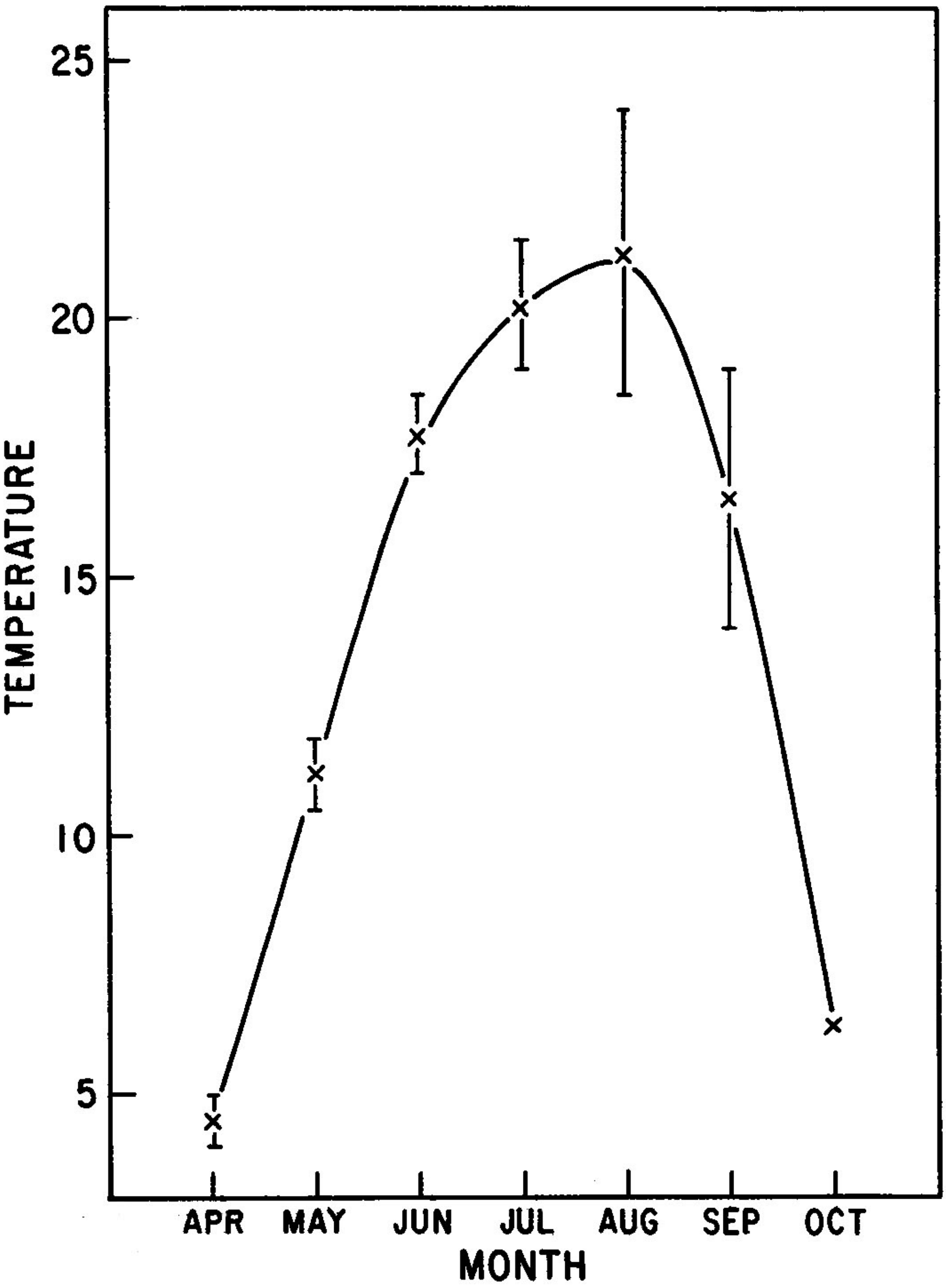

Fig. 3. Range in surface temperature at Barrachois from April to October, 1974.

mid-August. During 1974 a mean salinity of 25°/∞ for surface and bottom samples was determined for a period of 7 months (Fig 4). Similar observations were made from July to September, 1975, with a mean salinity of 29°/∞ being recorded.

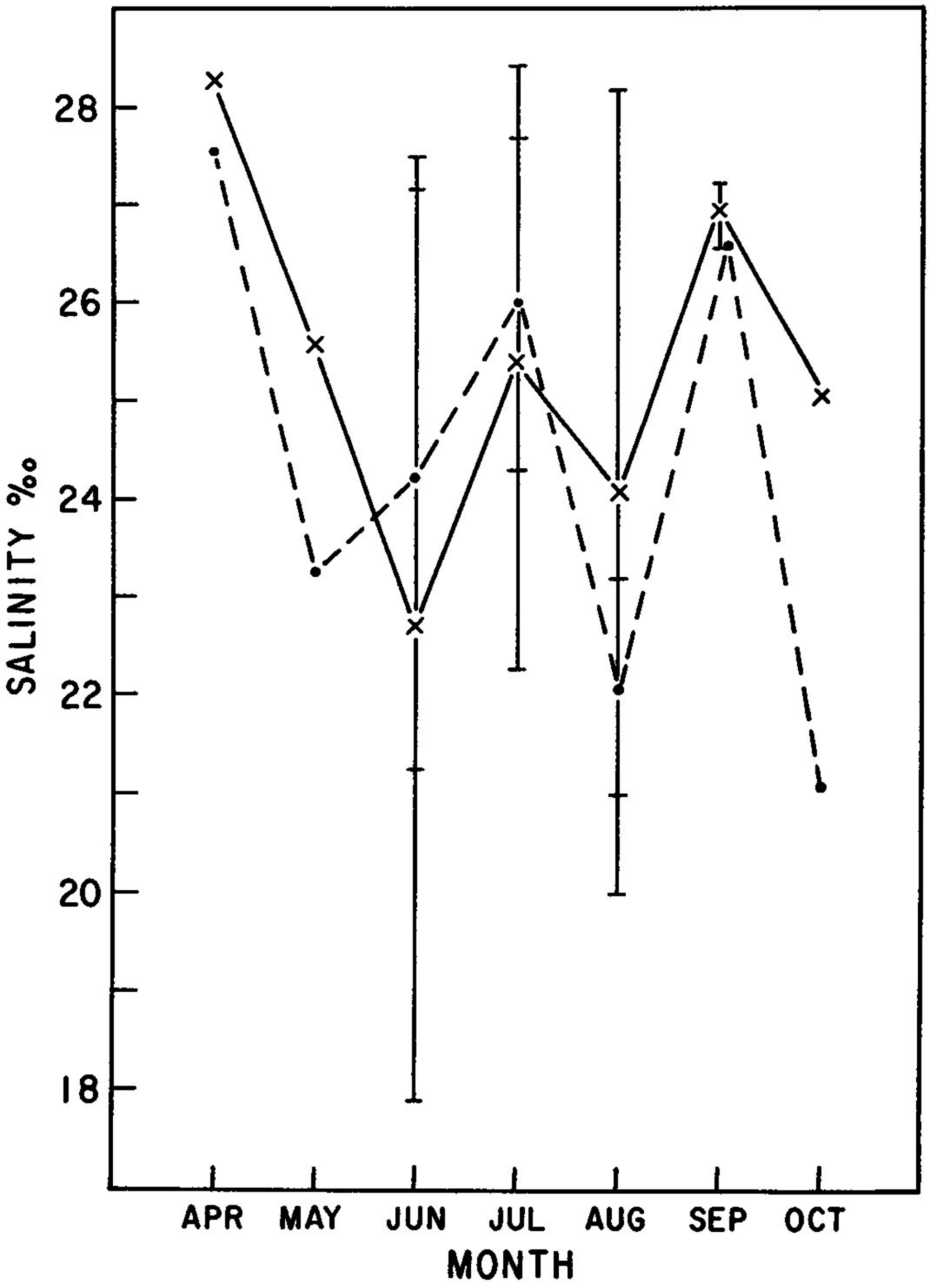

Fig. 4. Range in salinity of water samples taken at Barrachois from April to October, 1974. x———x surface-water sample; • — — • bottom-water sample.

Table I summarizes observations made on *Gracilaria* sp from June to September in 1974 and 1975. Reproductively mature tetrasporophytes, often arising from perennating stumps, were abundant in late June. By the end of July, both fertile sexual and asexual plants were releasing spores. Senescent plants were present by late August, although small, healthy plants were present in September.

Table I. Observations made on the population of *Gracilaria* sp at Barrachois, N.S. from June to September of 1974 and 1975.

Date	Observations
June	Presence of small, heavily epiphytized stump plants consisting of perennating thalli made detection of the population difficult; secondary erect shoots arose from the basal disc of these plants, and clean regenerated branches, often tetrasporic, also were seen.
Early to mid-July	Numerous healthy, bushy plants were present; plant size, although generally small, approached 10cm; reproductively mature tetrasporophytes and male gametophytes were present; sexually immature female gametophytes were also present; in 1975, many plants were found attached to the study line laid in July, 1974.
Late July to early August	Large, fertile sexual and asexual plants were collected; many of the plants were still reproductively immature.
Mid-August	Large, bushy fertile plants were seen; some apical decay was noted; cystocarpic plants were abundant.
Late August to early September	Many small plants were noted; presence of stump plants and large plants with decayed or necrotic upper branches indicated population senescence; majority of female plants were sexually mature; carpospore and tetraspore release still continued.
Late September	Numerous senescent, heavily epiphytized plants were seen; however, many healthy, clean plants also were present; and spore release continued.

In 1974 length of plants of *Gracilaria* sp increased from July to a maximum in early August, and then declined (Fig 5). A similar trend was displayed in 1975 (Fig 6). Density of plants was fairly low with a minimum in July increasing to a maximum in August, although distribution was relatively uniform (Table II).

A majority of plants in a population of *Gracilaria* was potentially capable of becoming reproductive with gametophytes being slightly more abundant than sporophytes. A reproductive maximum was recorded in early August, 1975, owing to increases in mature tetrasporophytes, carposporophytes and males. In late August, a decline in reproductive plants was noted, attributed to the decline in tetrasporophytes and males, with the proportion of carposporophytes remaining about the same (Fig 7). Contrariwise, in 1974 the ratio of infertile plants in late July and late August was similar. Despite the decline in tetrasporophytes in late August to about one-half the earlier value, the ratio of fertile plants did not change owing to the concomitant increase in carposporophytes (Fig 8).

At Mill River, P.E.I., fertility ratios were determined from samples of *Gracilaria* sp taken at depths of 0.5, 1.0, and 2.0 m. At the shallowest depth, the zone of *Gracilaria* began. Plants in this area were attached by holdfasts to shells or rhizomes of associated *Z. marina* or anchored either by byssal fibers of *Mytilus edulis* L. or by rhizomes of *Z.*

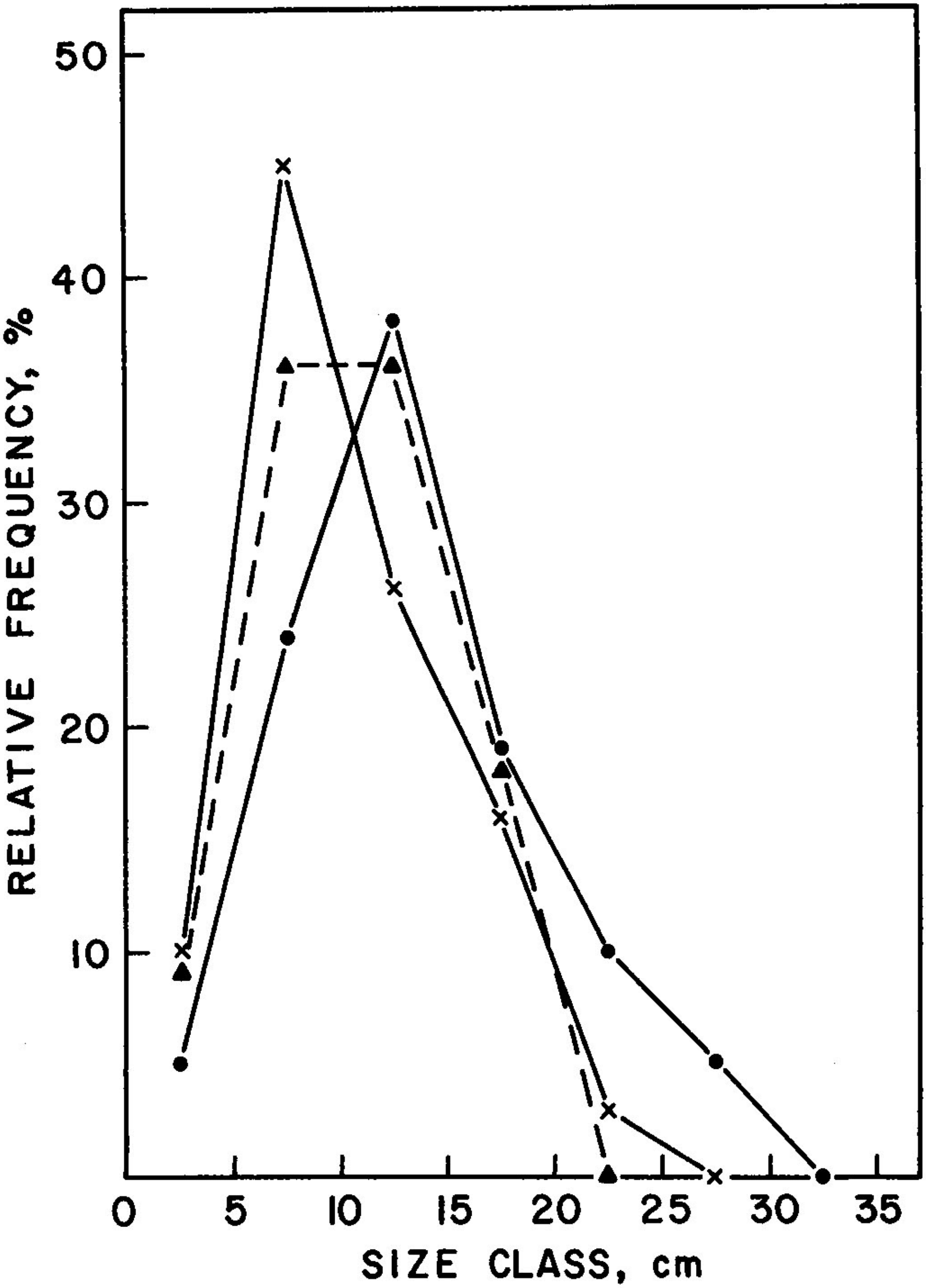

Fig. 5. Relative frequency of quadrats (m^2) containing *Gracilaria* sp of a maximum length in relation to size class at Barrachois during July and August, 1974. Points on graph represent class midpoints. x——x July 15th measurements of plants in 31 quadrats; •——• August 6th measurements of 21 quadrats; Δ — — Δ August 26th measurements of 22 quadrats.

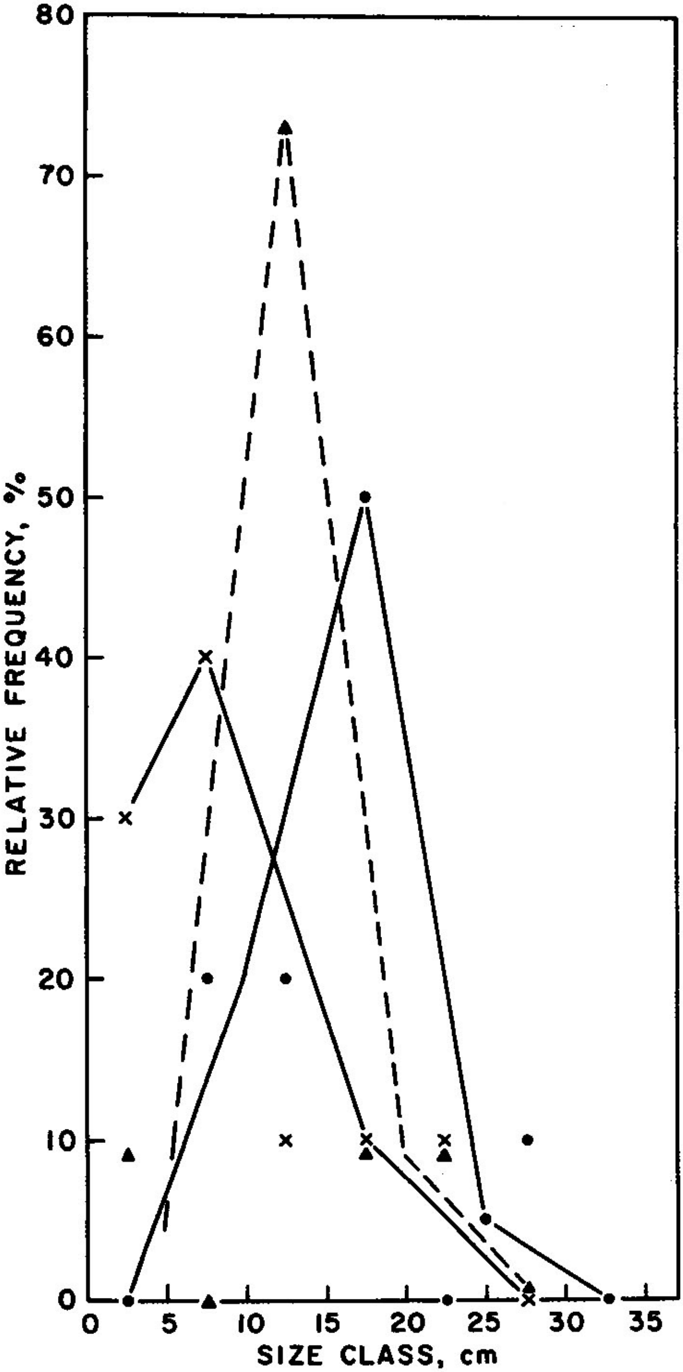

Fig. 6. Relative frequency of quadrats (m^2) containing *Gracilaria* sp of a maximum length in relation to size class at Barrachois during July and August, 1975. Points on graph represent class midpoints. x——x July 4th measurements of 10 quadrats; •——• August 5th measurements of 10 quadrats; Δ— — Δ August 28th measurements of 11 quadrats.

Table II. Density measurements of the population of *Gracilaria* sp at Barrachois during July, August and September of 1974 and 1975

Date	Area (m^2) sampled	Density (plants/m^2)	% Occurrence N/N t x 100
July 4, 1975	13	4	77
July 15, 1974	36	3	86
August 5, 1975	12	5	92
August 6, 1974	23	6	91
August 28, 1975	13	7	85
August 26, 1975	25	4	88
September 24, 1974	18	4	94

N = number of quadrats in which *Gracilaria* sp. occurred.
N_t = total number of quadrats.

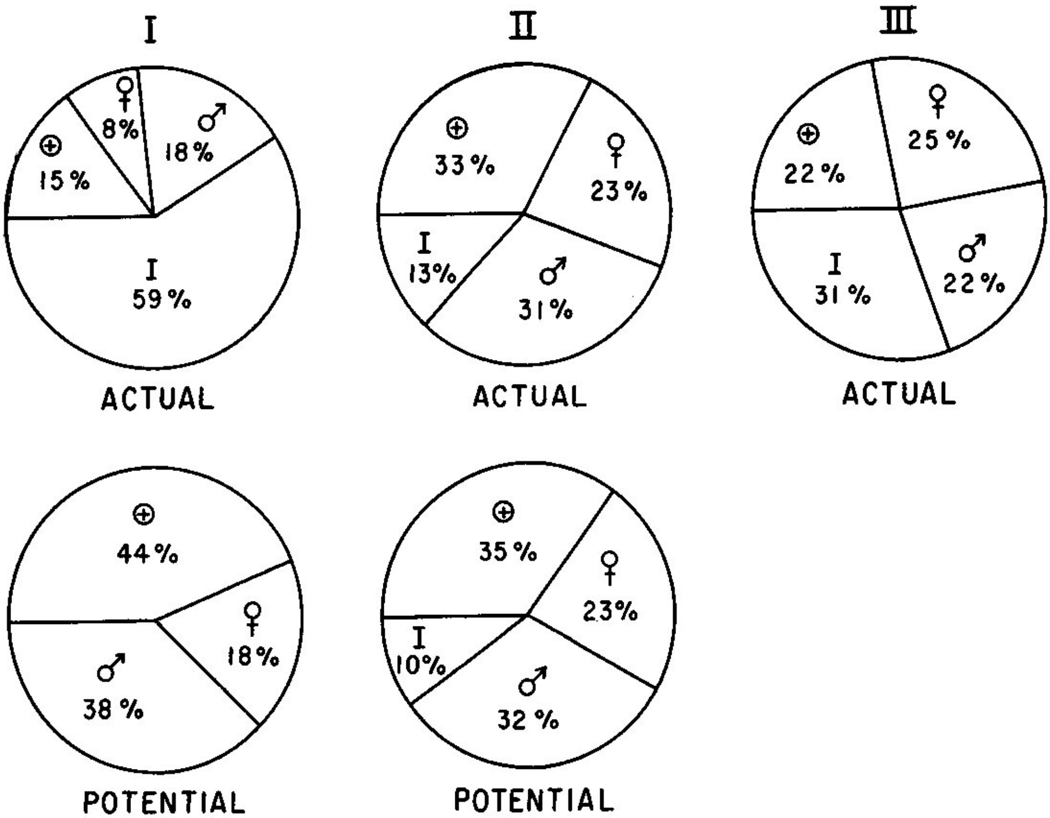

Fig. 7. Fertility ratios of 1975 population of *Gracilaria* sp at Barrachois Harbour. Actual = ratio obtained from excision of apical segments from attached plants (1 segment per plant). Potential = ratio obtained after laboratory incubation of these segments. ⊕ = tetrasporophyte; ♀ = female; ♂ = male; I = infertile; I = July 4th collection; II = August 5th collection; III = August 28th collection.

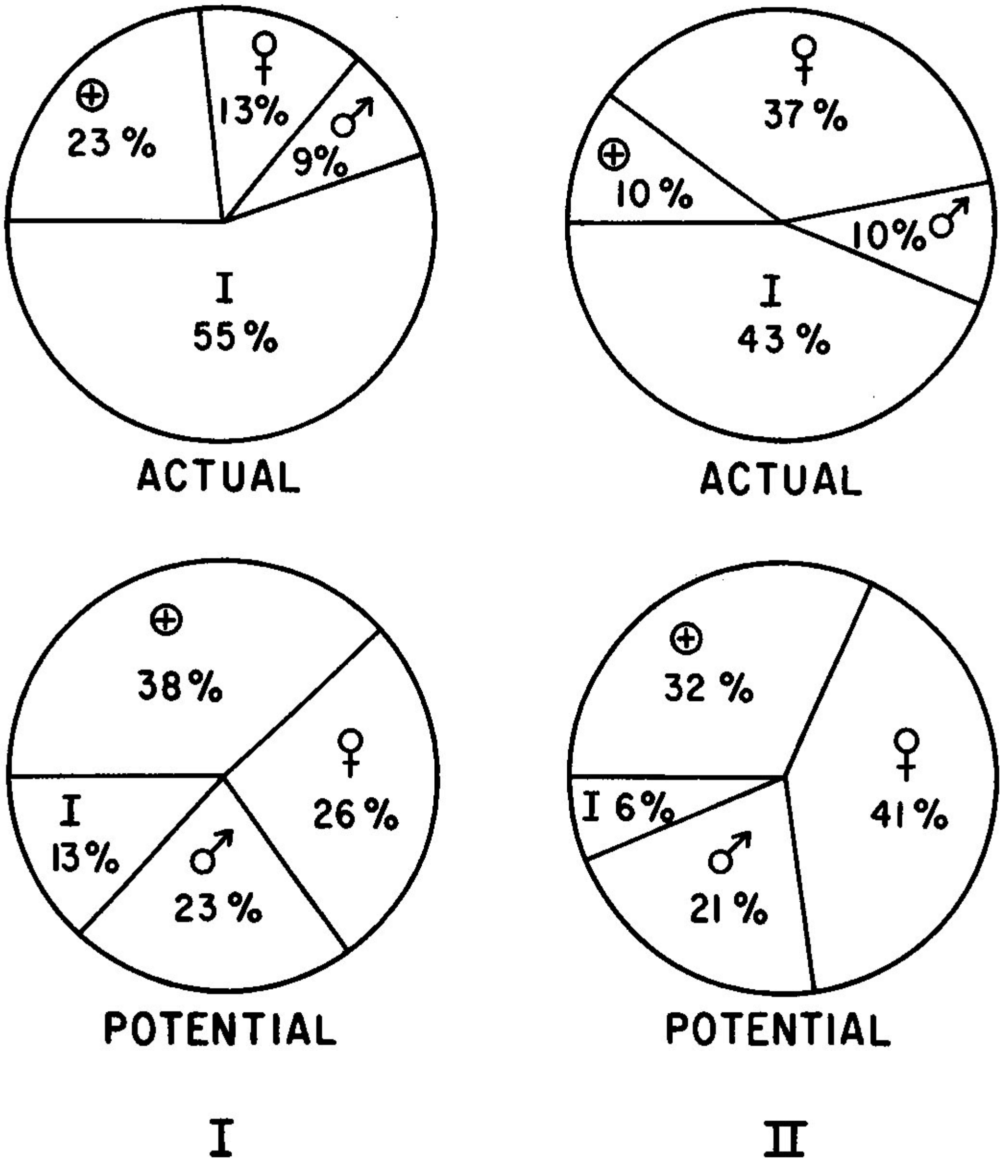

Fig. 8. Fertility ratios of 1974 population of *Gracilaria* sp at Barrachois Harbour. I = July 30th collection; II = August 26th collection. Symbols as in Fig. 7.

marina. *Gracilaria* sp, characterized by large bushy plants often having yellowish apices, was dispersed throughout the *Z. marina* zone.

At a depth of 1.0 m free-floating plants of *Gracilaria* sp had increased in quantity, as had other algal species such as *Ulva lactuca* L., *Sphaerotrichia divarcata* (C. Ag.) Kylin and species of Ceramiaceae. At a depth of 2.0 m a soft, muddy substrate prevailed in contrast to the firm, sandy bottom found at shallower depths. Only free-floating plants were found, occurring in dense clumps consisting of small fragments often entangled with one another by byssal fibers. Many of these plants were bleached in the apical region.

Only a small proportion of plants were attached by basal holdfasts at Mill River. Amongst these attached plants, gametophytes were more numerous than sporophytes (Fig 9). In contrast, free-floating or anchored plants consisted largely of fertile sporophytes, with a slight reduction in the percentage of fertile gametophytes. Also a greater proportion of detached plants was fertile compared to attached plants (Fig 9).

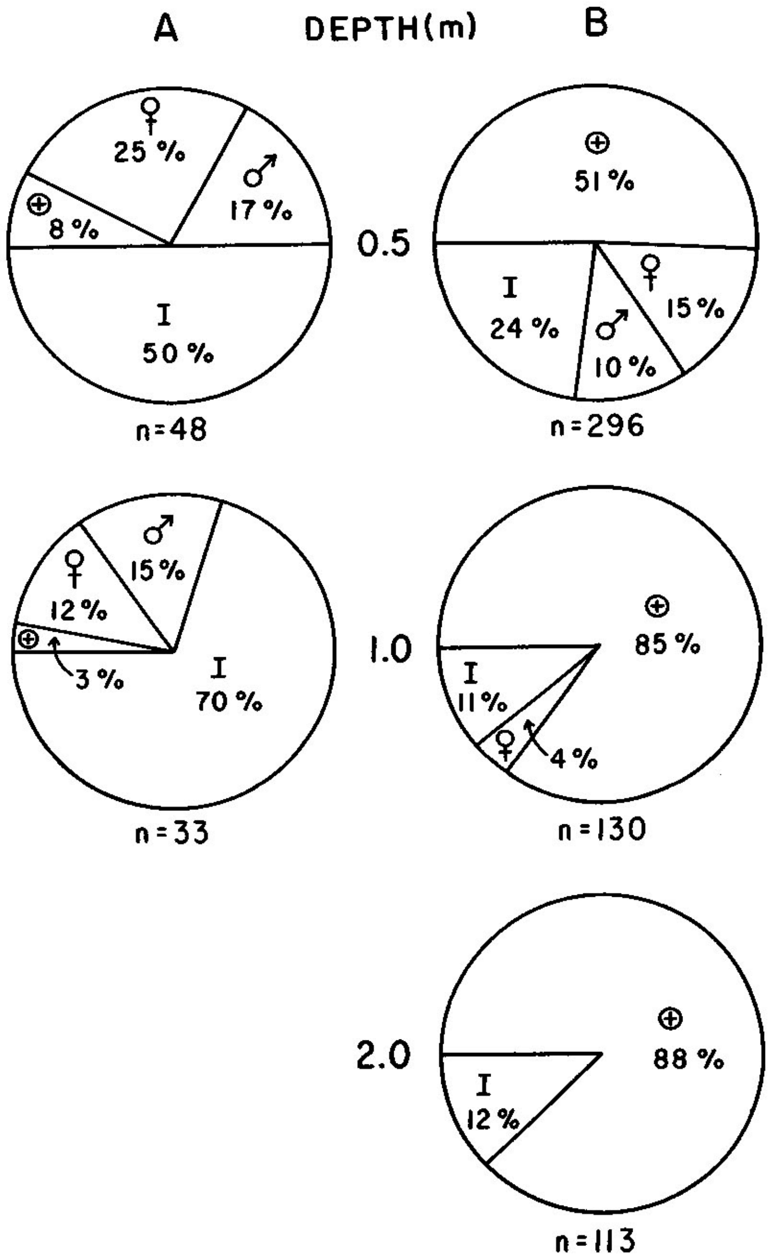

Fig. 9. Fertility ratios of populations of *Gracilaria* sp at Mill River in relation to depth, August 1, 1975. A = attached plants; B = anchored and free-floating plants; n = number of plants sampled. Symbols as in Fig. 7.

Branches with tetrasporangia were collected periodically; these released viable spores when cultured in the laboratory. From the end of June to the end of September in 1974 and 1975, viable tetraspores were released; viable carpospores were released from the end of July to the end of September.

In early June, 1974 sporelings of gametophytes were suspended in the zone of *Gracilaria* and in the channel at Barrachois. Plants in the zone of *Gracilaria* became fertile towards the middle of August after a field incubation period of 10 weeks. These plants attained a minimum length of 15 cm when sexually mature. Relative growth was highest during early August. After 12 weeks, maximum length was 20 cm. These plants were recovered in early November and only slight apical erosion in the upper branches indicated senescence. No differences in size were noted between upper and lower plants on the line. Sporelings transplanted in the channel attained reproductive maturity simultaneously with those in the zone of *Gracilaria*, but averaged only 14 cm in length in 12.5 weeks.

Gametophytic sporelings on shells nailed to the substrate in the zone of *Gracilaria* on July 23rd, 1974 became sexually mature in early September after a period of 6 weeks in the field. Length of plants had increased to 15 cm by the middle of August.

Transplanted sporelings on shells attached to cable were uniform in size although often epiphytized or covered with drift plants. In contrast, sporelings fixed to the bottom remained clean although variable in size, probably owing to silting. For example, after 6 weeks plant size ranged from 2.5 - 15.5 cm. Only plants 12 cm or more in length were fertile. Plants on shells nailed to the substrate overwintered successfully. The following year, however, growth was reduced owing to siltation. Ability of sporelings to overwinter was demonstrated by the presence in early 1975 of numerous large, fertile plants of *Gracilaria* growing attached to the study line.

Discussion

Shallow, warm-water embayments offer protection to free-floating algal macrophytes threatened in more exposed environments with exportation by swift tidal currents. Predominantly free-floating populations of *Gracilaria* occur in these areas where soft, mud bottoms are unsuitable for attachment and vegetative propagtion is favored over recruitment by spores. Attached members of the population are restricted to shallow regions stabilized by *Z. marina* where exposed pebbles and shells provide a firm substrate for attachment (C. Bird *et al* 1977a). However, only attached plants occur at Barrachois Harbour. Large fluctuations in tidal amplitude and strong channel currents prevented establishment of free-floating populations, and *Gracilaria* sp was limited to a narrow shelf protected by *Z. marina* immediately above.

Turbidity reduced light intensity in the zone of *Gracilaria*. However, transplanted plants grew well, both in the zone of *Gracilaria* and in the channel, suggesting that increased hydrodynamic force and instability of the substrate, rather than levels of low light, prevented establishment of the population in the channel.

Constant tidal flow ensured uniform mixing throughout the water column, and prevented vertical salinity and temperature stratification. Growth, enhanced by increased water movement, was rapid during summer months when solar radiation, daylength, and temperature reached maximum values; plants transplanted in late July showed a 4-week reduction in time required for reproductive maturation compared to plants transplanted in early June when water temperatures had not yet reached 20°. Sporelings outplanted in Pomquet Harbour, Nova Scotia (C. Bird *et al* 1977b) also demonstrated that July and August were the most favorable months for growth and reproductive maturation; sporelings outplanted in June became fertile after 10-16 weeks in the field whereas those outplanted in July and August became fertile in 4-8 weeks.

The growing season was less than 3 months. During this period plants matured with a reproductive peak observed in early August. Early maturation of tetrasporophytes in late June enabled release of spores to continue throughout the growing season. Transplantation experiments suggested that there was sufficient time for tetraspores released in early summer to develop into mature gametophytes by late August, a possible explanation for the greater percentage of gametophytes in August, 1974 (Fig 8). Male plants were abundant by mid-July. Contrariwise, in many attached populations of *Gracilaria*, male plants have been reported as scarce or reduced in number compared with female and tetrasporic plants (Jones 1959; Kim 1970; Phillips 1925; Rao 1973). Reproductive peak of carposporophytes was recorded in early August; a longer maturation period being required owing to the necessity of fertilization. Periodicity of production of spores has been noted in several populations of *Gracilaria* spp (Jones 1959; Kim 1970; Rao and Thomas 1974; Saito 1959; Simmonetti *et al* 1970; Stokke 1957), peaks being recorded during seasons of maximum photoperiod and temperature of the water (Jones 1959; Kim 1970) when growth was optimum.

There was insufficient time for sporelings originating from carpospores to reach maturity before water temperatures declined, thus probably limiting growth. However, rapid growth of sporelings reduced the threat of burial and increased chances of survival at low temperatures.

Senescence of the population was noticed following reproductive maturation of carposporophytes. Upper fertile branches of plants eroded resulting in stump plants that overwintered in a state of low metabolic activity. Evidence of necrosis was reported in late August for the free-floating populations at Pomquet Harbour (C. Bird *et al* 1977b). In both populations growth resumed upon increasing light and temperature in the spring.

Thallus perennation was important for survival of the attached population at Barrachois and the predominantly free-floating population at Mill River. A major difference between these populations was the mode of reproduction; the free-floating population, although dominated by tetrasporophytes, depended on vegetative propagation whereas the attached population reproduced via spores and exhibited an alternation of generations.

Population of *Gracilaria* at Barrachois demonstrated a *Polysiphonia*-type life history as shown in laboratory culture (N. Bird *et al* 1977). At Barrachois, gametophytes and sporophytes were abundant with the slightly greater proportion of gametophytes. This is attributed to germination of tetraspores early in the growing season and delayed senescence of gametophytes until late August while less noticeable decay of tetrasporophytes was in progress. Hansen and Doyle (1976) suggest from results of population studies on *Iridaea cordata* (Turner) Bory, that the observed dominance of the tetrasporophyte over the gametophyte is an adaptive response of the life history phase. These workers suggest that this disproportionality may occur in an environment where a majority of tetraspores is unable to germinate and establish a gametrophytic phase, a plausible explanation for observed reproduction behavior of *Gracilaria* sp at Mill River, P.E.I. In addition, carposporophytes are more susceptible to decay than sporophytes (Jones 1959). In comparison, tetrasporophytes suffer only minor loss during the overwintering phase and can resume growth quickly in the spring.

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